SURFACE MODIFICATION FOR BIOCOMPATIBILITY

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Figure 7: The effect of artificial surfaces on neurotransmitter phenotype expression of embryonic cortical cells cultured in serum-free medium. Fluorescence photomicrographs of GABA and glutamate- immunoreactive cells cultured for 7 days from E14 rat cerebral cortex. Sister cultures have been incubated with anti-GABA and anti-glutamate antibodies. On poly-D-lysine and DETA, glutamatergic neurons represent approximately 30 - 40% of total cells, while GABAergic neurons represent about 50 - 60% of total cells. The ratio of glutamatergic to GABAergic neurons is 0.63 : 1. On 13F, about 20 - 30% are composed of glutamatergic, 50 - 60% express GABA, the ratio of glutamatergic to GABAergic cells is 0.45 : 1. Results suggest that the hydrophilic surface (DETA), simular to poly-D-lysine, supports transmitter phenotype expression, while the hydrophobic surface (13F) decreases the number of glutamatergic cells, but has no significant effect on GABAergic cell expression.

Figure 8: Influence of bFGF on survival and leading edge extension of cortical astrocytes cultured in serum free medium for 4 days. Astrocytes were grown on poly-D-lysine (PL), DETA, 13F, and OTS and immunostained with anti-GFAP. Fluorescence photomicrographs show higher cells survival and leading edge extension of GFAP+ astrocytes on PL and DETA than on 13F and OTS. The addition of bFGF improved cell survival and growth on 13F and OTS, but did not significantly affect cell growth on PL and DETA, suggesting that bFGF exerts a protective effect on astrocytes *in vitro*.

Figure 9: Phase (1,3,5) and fluorescence (2,4,6) microscopy showing microglial cultures and differential adhesion according to substrate. Both ameboid and activated stages of microglia are present on control poly-D-lysine (1,2,3,4), with diminished attachment on DETA substrates (5,6).

Figure 10: Fluorescence photomicrographs showing cortical microglial cultures plated on poly-D-lysine, DETA and 13F after 12 days in culture, fixed and immunostained with microglial specific marker OX-42 (Serotech). Many of the microglia project thin processes and resemble ramified microglia. DETA, similar to poly-D lysine in hydrophobicity, promotes survival and growth. A more hydrophobic surface, 13F, elicits poorer attachment/survival.

Figure 11: XPS survey spectra. APTS monolayer (bottom). APTS monolayer derivatized with covalent crosslinker, glutaraldehyde (middle). APTS monolayer with covalent crosslinker glutaraldehyde attached to apo-transferrin (top). The X-axis shows the binding energy of electrons in the film and is directly related to different elements. The Y-axis shows the relative XPS intensity. Note that the size of the C1s and N1s signal as compared to the size of the Si2p signal and is indicative of the amount of bound material after reaction and washing to remove loosely bound material.

Figure 12: Panel of phase images of E18 rat cortical neuron cultures after 4 days in culture in MEM + N3/g + 5 ng/ml bFGF on APTS monolayers either modified by apotransferrin (APO) or bovine serum albumin (BSA) adsorbed to the surface or derivatized with covalent crosslinker, glutaraldehyde (GLU). Controls are shown here: poly-D-lysine (A), APTS (B), and APTS + GLU (C).

Figure 13: Panel of phase images of E18 rat cortical neuron cultures after 4 days in culture in MEM + N3/g + 5 ng/ml bFGF on APTS monolayers either modified by apotransferrin (APO) or bovine serum albumin (BSA) adsorbed to the surface or derivatized with covalent crosslinker, glutaraldehyde (GLU). APTS + APO (A); APTS + GLU + APO (B); APTS + BSA (C); APTS + GLU + BSA (D). Controls were APTS, APTS + GLU, and poly-D-lysine.

Project Summary

The purpose of this project is to develop modified artificial surfaces for implantable biocompatible electrodes in the cerebral cortex. To this end, primary objectives of the project are to develop an *in vitro* cell culture model and to use *in vitro* cultures to select surface modifications that control the interaction of neurons, astrocytes, and/or microglia in embryonic and adult stages of development. This Quarterly report will summarize the results and conclusions of the project to date. In brief we have:

1) established an *in vitro* system that mimics the environment found in CSF and studied cortical neurons, astrocytes and microglia in this system.
2) Demonstrated embryonic cells (E12-E14) will exhibit adult markers in our *in vitro* system (after 7-14 days) and compared these results to adult cells in the same system.
3) Demonstrated surface analysis is a key component in understanding the results, as well as provide a quantitative measure of system performance.
4) Showed that surface composition can be used to select for neuronal phenotype.
5) Beginning preliminary *in vivo* experiments.

Overall project objectives

a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system/
b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures:
c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise: A 16 channel CMOS neuronal stimulating array *(IEEE Trans. Sol State Circuits* 27: 69-75, 1992) and to chemically characterize these surfaces before and after protein adsorption.

1. The attachment method shall be stable in saline at 37°C for at least 3 months;
2. To use silane coupling as the method of attachment;
3. To use the silanes to control the spatial extent (i, the pattern) of the deposited surface.

d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces:

e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

Background

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microelectrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro-environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to promote cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

RESULTS

Self-Assembled Monolayer Modifications

To prepare biocompatible surfaces for neuronal cell survival and axonal outgrowth we modified different materials with self-assembled monolayers (SAMs). Three of the substrate materials that have been modified are silicon, glass and silicon nitride. These materials are cleaned prior to modification to ensure the removal of surface contaminants and to create reactive silanol groups on the substrates surface. These surface silanol or hydroxyl groups are used as a final reactive site for preparing SAMs. A solution of reactive silanes such as mono-, di-, or trichloro (R-Si-$(Cl)_x$ X=1,2,3) or trimethoxy (R-Si-$(OCH_3)_3$) silane. (Where R is a tethered functional group such as an amine, methoxy, thiol, etc.) Minimization of water is very important for this reaction not to occur too quickly, however a very small amount is necessary to make the reaction occur. A very thin adsorbed water layer on the surface of the substrates makes the chloro or methoxy groups hydrolyze to form R Si $(OH)_x$. The R-Si-$(OH)_x$ molecules will hydrogen bond with each other and/or to the surface. At this point, condensation can occur creating a covalent bond with the surface silanol or hydroxyl group. After repeated rinses of the surface to remove unbound silanes, a final heat step causes further condensation driving final Si-O-Si bonds to form and completing the self-assembly. The SAMs provide an effectively new surface whose chemical properties may be controlled by careful choice of silanes containing selective chemical functional groups.

The matrix of SAMs that have been examined for cell culture survival and axonal outgrowth are shown in Table 1. The average water advancing contact angles are given for each monolayer prepared in descending order starting with the most hydrophobic surface. The contact angle measurement is a good representation of the hydrophobicity of the surface. 13F is the most hydrophobic surface shown with an average contact angle of 103 degrees. Neuronal survival is not only based on some critical amount of hydrophobicity but also on the functional groups presented by each monolayer.

Surface Analysis → Before Culture

Surface analysis by X-ray photoelectron spectroscopy (XPS) is necessary for this program in the same way that an NMR spectrometer is necessary for conducting an organic synthesis program. Since we are synthesizing new surfaces and modifying their properties, we need to assay the result of the surface before (starting material) and after (reaction product) modification. This is analogous to examining the synthesis of new chemical by NMR. Figures 1 and 2 show N 1s spectra for several representatives of the

SAMs we have used to date to optimize cell culture response to the surfaces. The nitrogen is indicative of the presence of an amine functionality as well as the degree of protonation (high energy vs. low energy peak). This is important because we have seen a direct correlation between degree of protonation and cell viability.

Table 1

Abbreviation	Chemical	Contact angle, θ_{adv}
OTS	octadecyltrichlorosilane	103
NDEC	N-decyltrichlorosilane	98
13F	(tridecafluoro-1,1,2,2-tetrahydrooctyl) 1 dimethylchlorosilane	90
TCMD	(10-Carbomethoxydecyl) dimethylchlorosilane	67
PPDM	(3-phenylpropyl)dimethylochlorosilane	64
CP	3-cyanopropyldimethlchlorosilane	63
TP	triphenylchlorosilane	60
PEDA	(aminoethylaminomethyl)phenethyltrimethoxysilane	56
MTS	3-mercaptopropyltrimethoxysilane	54
MTS-OX	oxidized 3-mercaptopropyl-trimethoxysilane	46
NBUT	n-butyldimethylchlorosilane	44
APTS	aminopropyltrimethoxysilane	42
DETA	trimethoxysilylpropyldiethylenetriamine	37
EDA	N-(2-aminoethyl)-3-aminopropyltrimethoxysilane	35
MAP	N-methylaminopropyltrimethoxysilane	34
D-MAP	(N,N dimethyl-3-aminopropyl)trimethoxysilane	31
PEG-350	triethoxysilyl polyethyleneglycol (mw = 350)	30
UAD	11- undecanoic acid dimethylsiloxy	21

Stability of SAMs

For use *in vivo*, it is important to assess the stability of the SAMs under physiological conditions. Initially, eight (8) SAMs were tested on glass coverslips in PBS at 37 degrees. With the possible exception of the MTS SAM, there was evidence of a significant decrease in molecular coverage after two weeks. This can be attributed to hydrolysis, as has been reported by other workers.

To approach more realistic conditions, a second test included a buffered solution of protein using the concentration found in our *in vitro* serum-free cultures (bovine serum albumin, BSA, 0.001%). For the highly-fluorinated 13F-Cl_1 monolayer, the XPS data were modeled to allow for the attenuation of the F signal by a rapidly deposited protein overlayer that adsorbs when BSA is present in solution. This model supported that in the presence of an adsorbed adlayer some silanes are much more stable. We hypothesize that the protein layer inhibits hydrolysis of SAM molecules from SiO_2. This provides a basis for understanding the long-term effects of surface monolayers on cell culture systems.

where proteins are part of the medium and are produced by the cells as well. However, after 4-8 weeks, we observed a further decrease in the F signal but also the increase of ions (Ti, Na, ..) that are indicative of glass dissolving. In further experiments, the stability of trichloro- and monochloro-terminated 13F silane monolayers was tested on two different substrates, glass cover slips and silicon wafers in a higher concentration of BSA (0.1%). Interestingly the trichloro 13F monolayer (on SiO_2) was found to be more stable than the monochloro 13F for short periods.

Taken together, these studies show that the adsorbed proteins expected in any real biological evaluation stabilize the SAM for an initial period. The reaction mechanism of the SAM with the surface is also a key element, and as indicated by the greater stability of groups with multiple attachment points (trichloro vs. monochloro). The glass is unstable in the presence of PBS but we have used results from another project to show we can stabilize the glass and then resultant monolayers. Finally, since the ultimate goal is for the SAM to just be a template for initiating the incorporation of an implant it may be enough to have short term stability.

Post-mortem XPS of cell culture plates

The amount of material deposited on the surfaces by the cell culture process has been measured by XPS. Figure 3 compares XPS peak areas for DETA with and without cells, and indicates that the deposited material is from the cells and not from the surrounding serum free medium (MEM/N3). This is extremely important for determining the response of cells in a defined *in vitro* system. In addition, Figure 3 shows that in the presence of cells a protein layer is rapidly established but then levels off with time. Good agreement is found in the amount of protein for DETA and PL, which also showed similar cell morphology and attachment. Modeling of the normalized C 1s peak areas yields a protein layer thickness of 60 to 70 angstroms for both surfaces after 10 days in this medium.

The examination of the deposited material after culture has proved to be very informative in diagnosing the cells' response to surfaces and correlates with morphology. We have shown that in healthy cell cultures, in our model system, that the deposited material increases over a one to three day period and then stabilizes. Conversely, on surfaces where cells display undesirable morphological features such as clumping, necrosis, and cell death, the layer continues to increase until the end of the culture. We are preparing a manuscript to detail these observations.

Cell Morphological and Immunostaining Results:

Adult cultures

We have successfully cultured adult rat cortical cells in a defined, serum-free culture (Figure 4). This presents an extraordinary opportunity to relate embryonic and adult *in vitro* results and then compare them to the *in vivo* results. We have proven there are neurons by showing that dissociated cells express the appropriate neuronal markers, an example of which is microtubule associated protein (MAP 2) which is shown in Figure 5. This *in vitro* system will allow us to analyze adult cortical neuron response to injury, to implantable substrates and allow us to formulate conditions in a defined culture environment toward development of effective *in vivo* implantation.

Phenotype determination

We also investigated the effects of SAMs on cell phenotype expression of rat cerebral cortex in a defined system which is particularly important if our hypothesis that the body can be directed to incorporate the implant is valid. Cell lineage studies have shown that there are two major types of neurons: pyramidal and non pyramidal cells (Price and Thurlow, 1988; Parnavelas et al., 1992) that have been identified in the rat cerebral cortex. Pyramidal neurons use the excitatory transmitter glutamate whereas most non-pyramidal neurons use the inhibitory neurotransmitter GABA (Jones and Hendy, 1986; Emson and Lindvall, 1989). By using antibodies against glutamate and GABA we are able to identify these two types of neurons. There are also three distinct types of glial cells: astrocytes, oligodendrocytes and microglia (Grove et al., 1993). Using specific cell markers we can detect each of those types of glial cells. All the neurons and glial cells that comprise the adult cerebral cortex are derived from the embryonic precursors. Cell phenotype choice to become neurons or glia and neurotransmitter choice to become glutamatergic or GABAergic are a crucial step in cortical development. The factors that control the timely differentiation of the precursor cells into differentiated cell types are not well known. It is believed that the cell environment plays a key role in the specification of cortical cells, even though a cell's intrinsic developmental program is important in regulating cell lineage (Williams and Price, 1995). In our studies, the expression of cortical cell phenotype was examined in an *in vitro* system in which embryonic rat cortical cells were grown on silica substrates modified with artificial surfaces composed of silane self-assembled monolayers (SAMs) in serum-free medium (Figure 6). Sister cultures were immunostained with a panel of antibodies to detect specific differentiation markers: neurons and glial cells are identified by using rabbit anti- NSE (neuron- specific enolase, 1:5000, Chemicon) and mouse anti-

GFAP (glial fibrillary acidic protein, 1:500, Sigma) pyramidal and non-pyramidal neurons are distinguished by using guinea pig anti-GABA (1:300, Chemicon) and mouse anti-glutamate 1:500, Inestar).

Double-immunofluorescence staining for NSE and GFAP of cultured cortical cells dissociated from E17-18 rats showed that GFAP+ cells (astrocytes) appeared about 7 days in culture and increased with age while NSE+ cells (neurons) were reduced. In older cultures, more astrocytes relative to neurons were detected. On hydrophobic surfaces (13F and OTS), fewer neurons were found than on a hydrophilic surfaces.

Double-immunofluorescence staining for glutamate and GABA was carried out in sister cultures (Figure 7). Results showed that in cultures grown on poly-D-lysine, glutamatergic neurons represent approximately 30 - 40% of total cells, GABAergic neurons represent about 50 - 60% of total cells, and the ratio to glutamatergic to GABAergic cells was 0.6 : 1, which is consistent with finding of immunocytochemical studies *in vivo* (Conti et al., 1987; 1989). In cultures on DETA, this ratio was 0.60 : 1. In contrast, on 13F and OTS, about 20 - 30% were composed of glutamatergic, 50 - 60% expressed GAD and the ratio of glutamatergic to GABAergic was 0.45 : 1. This suggests that a hydrophilic surface (DETA), similar to poly D-lysine, supports both glutamatergic and GABAergic transmitter phenotypes, while hydrophobic surfaces (13F and OTS) decrease the number of glutamatergic cells more than GABAergic cells. This experiment indicates we can use surface chemistry to select for cell phenotype.

Another striking finding was from our studies on effects of a combination of two factors (substratum and growth factor) on cell survival and differentiation. We investigated the effects of bFGF on cell survival and process extension of astrocytes taken from new born rat cerebral cortex grown on artificial surfaces (Figure 8). The leading edge extension is characterized by the leading edge output, the branch nodes and the number of arbors. Results showed a striking trophic action of bFGF on astrocytes grown on poor surfaces (13F and OTS), but not cells on poly-D-lysine and DETA (1-4) These findings strongly suggest that bFGF exerts some protective effect on astrocytes *in vitro*.

In another set of experiments we investigated the effects of bFGF, BDNF, and CNTF on neuronal survival and neurite outgrowth on artificial surfaces. The cortical cells were grown on poly-D-lysine, DETA, 13F, and OTS. The cell survival was assessed by a comparison of the number of cells surviving a given point relative to the number of cells surviving at the time of culture initiation. The extent of neurite outgrowth was characterized in terms of total process output, the mean arbor output, the number of arbors and cell body area. Results showed that hydrophilic surface (DETA), similar to poly-D-lysine, supported the growth of primary cortical neurons, whereas poor attachment and

consequent poor cell survival and neurite growth occurred in cultures grown on hydrophobic surfaces (13F and OTS). However, the addition of bFGF (20ng/ml), BDNF (100ng/ml), and CNTF (20ng/ml) increased the number of cells surviving and neurite growth to various extents. Double immunofluorescence staining with a mixture of anti-glutamate (Incstar) and anti-GAD (1440m gift of Dr. Kopin) in sister cultures showed that the growth factors enhanced cell survival and neurite outgrowth for both glutamatergic and GABAergic neurons. This study suggests that bFGF, BDNF and CNTF have a protective effect for neurons, *in vitro*, against poor substrata.

Microglia

We have preliminary results indicating substrate can play a key role in microglial attachment, activation and response (Figures 9, 10). Figure 10 shows microglial cultures at day 12, fixed and stained with OX-42 (1:100 Serotech). Microglia plated on poly D-lysine controls and DETA have improved attachment and survival as compared to cells plated on the 13F monolayer. The cultured microglia project thin processes and resemble ramified microglia (Figure 9, 10). Microglial cultures of rat have proved to be difficult which decreases the amount of usable data, but we are working on alternative preparations.

Covalent Attachment of Biological Macromolecules

SAMs play an important role in the preparation of a reproducible functionalized surface. SAMs can also be used to covalently attach biological macromolecules to the surface. The matrix of SAMs as shown in Table 1, provide a number of different functional groups to do further chemistry. Covalent attachment chemistry to SAMs are done with crosslinkers such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), phenyldiisothiocynate, N-succinimidyl 4-maleimidobutyrate (GMBS), or glutaraldehyde.

Several homogeneous cross linkers have been surveyed for protein attachment. Two of these are phenylene diisothiocyanate and diethylmalonimidate. These were found to be less effective for cross linking apo-transferrin than glutarldehyde. Figure 11 shows XPS characterization of the attachment of apo-transferrin to an APTS monolayer. The bottom spectra in this figure shows the APTS monolayer. The underlying glass substrate is detected by the predominance of silicon (Si 2s peak at 150 eV, Si 2P peak at 100 eV) and oxygen (O 1s peak at 530 eV, Auger peak at 970 eV) as expected. APTS attached to this substrate is characterized by the carbon (C 1s peak at 280 eV), and nitrogen (N 1s peak at 400 eV). An increase in the carbon signal (middle spectra) is seen after reaction of glutaraldehyde with APTS. The substrates were copiously rinsed of glutaraldehyde with

DI water and immediately placed into a solution of apo-transferrin. The protein attachment step in this procedure is followed by many washes of a surfactant in PBS to remove any unreacted protein on the surface. The covalently attached apo-transferrin is shown in the top spectra by an increase in both the nitrogen and the carbon peaks relative to the silicon response.

In addition to apo-transferrin (APO), bovine serum albumin (BSA) was also covalently attached to an APTS monolayer for survey of cell culture to E 18 rat cortical neurons cultured in MEM + N3/g in the presence of 5 ng /ml bFGF. Figures 12 and 13 show the contrast of the covalently attached protein to the adsorbed protein with additional controls (i.e., PDL, APTS, APTS + glutaraldehyde(GLU)). While the outcomes are preliminary and subtle; survival, process outgrowth and cell population expression are modified by the presence of proteins: both adsorbed and covalently linked BSA (2 C,D) and covalently linked APO (2 A,B) as early as day 4.

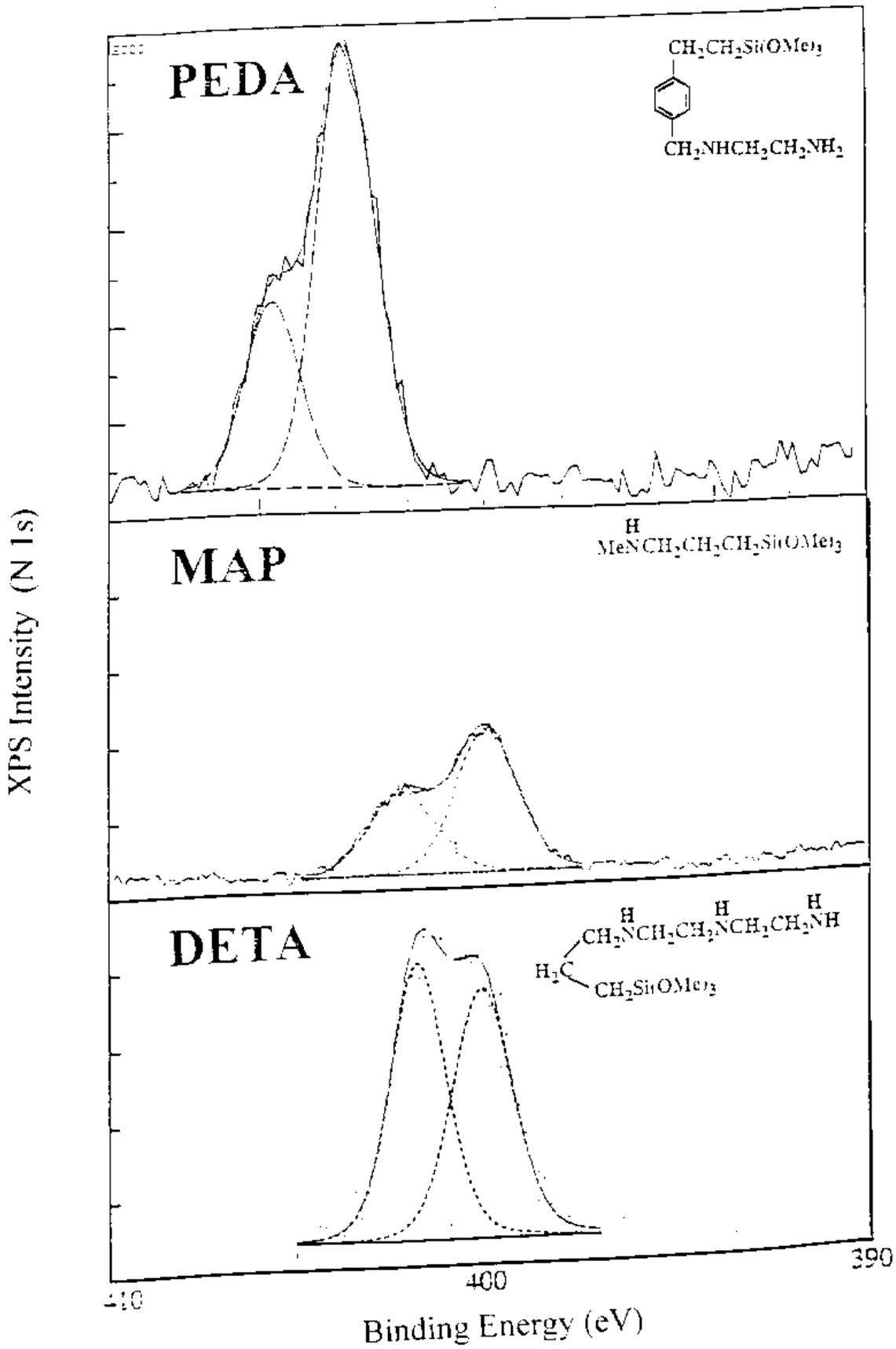
Additional survey experiments were done on a series of covalently attached biological macromolecules. The water soluble carbodiimide crosslinker 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), was used to attach the proteins laminin and fibronectin and glycosaminogyleans heparin sulfate and chondroitin sulfate. This crosslinker under acidic conditions forms amide bonds between an amine (from a monolayer such as APTS or DETA) and a carboxylic acid functional group (from the biological macromolecule).

Collaborations

We established a collaboration with W. Agnew at the Huntington Medical Research Institute. While we have not yet specifically modified electrodes, we have successfully modified the poly-silastic sheath that holds a series of microelectrodes in place along the spinal cord during implantation (Agnew et al., 1990). One problem we are focused on solving is that the sheath has glial scar buildup and adhesions that eventually displace the electrodes laterally (with adhesion) and vertically (due to buildup of tissue). We have contacted Dr. Albert Lossingsky and will begin a new series of experiments once the costs of the *in vivo* work is determined and Dr. Agnew's approval of the scope of the project is obtained. This has taken much longer than we had anticipated but we are hopeful to generate at least some data before the end of the project.

In addition to collaborations with the Huntington Institute, we have contacted and are in the process of establishing studies of *in vivo* implantation of modified materials into cortex in animal models with Cordell Gross at the University of Vermont and Sharon Juliano At Uniform services University of the Health Sciences in Bethesda.

Figure 1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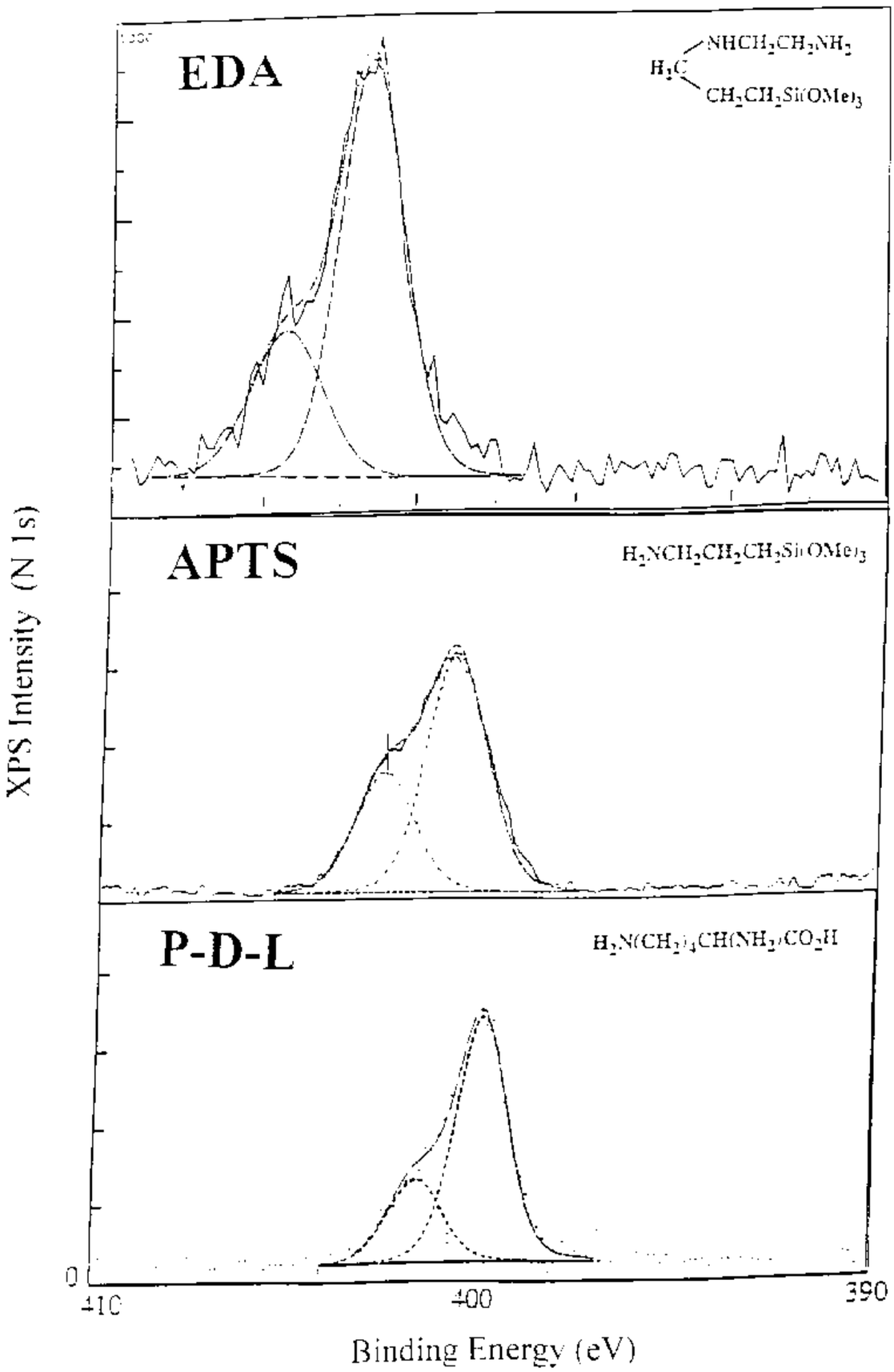
MAP
H
$MeNCH_2CH_2CH_2Si(OMe)_3$
DETA
H H H
$CH_2NCH_2CH_2NCH_2CH_2NH$
H_2C
$CH_2Si(OMe)_3$
XPS Intensity (N 1s)
410
400
390
Binding Energy (eV)

Figure 2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APTS
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XPS Intensity (N 1s)
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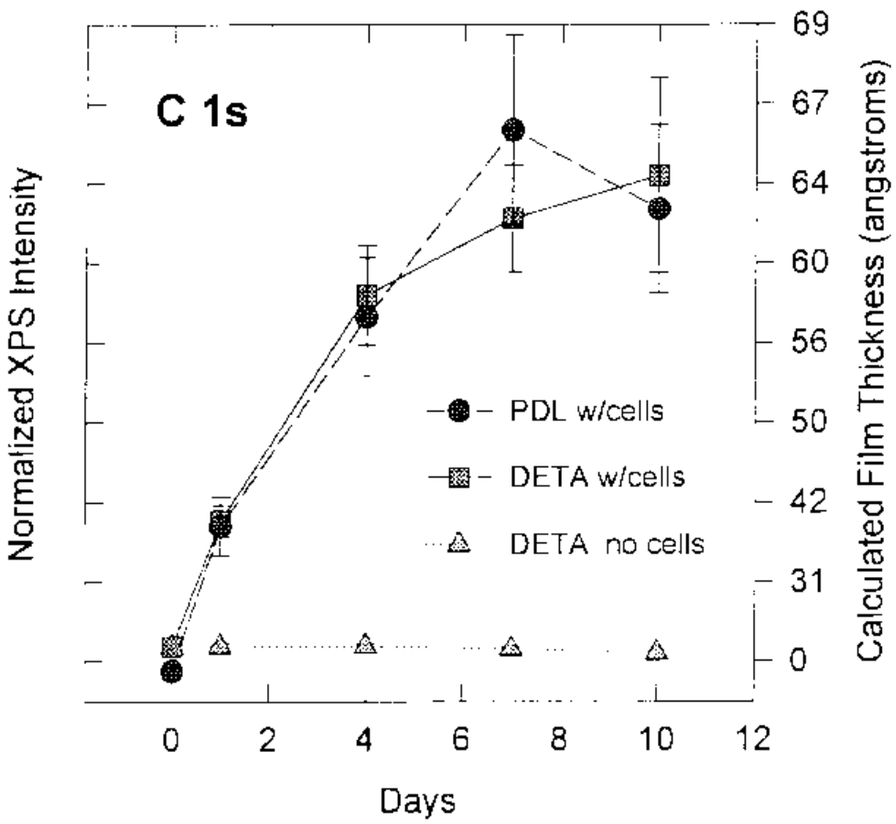
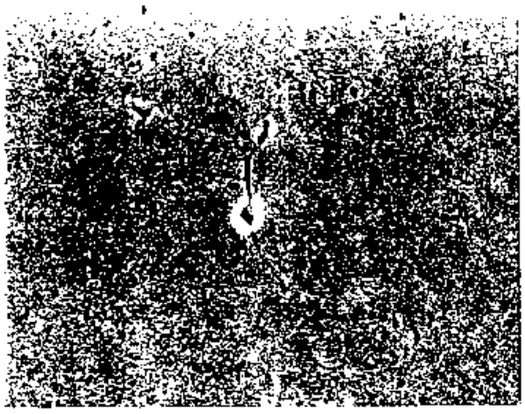
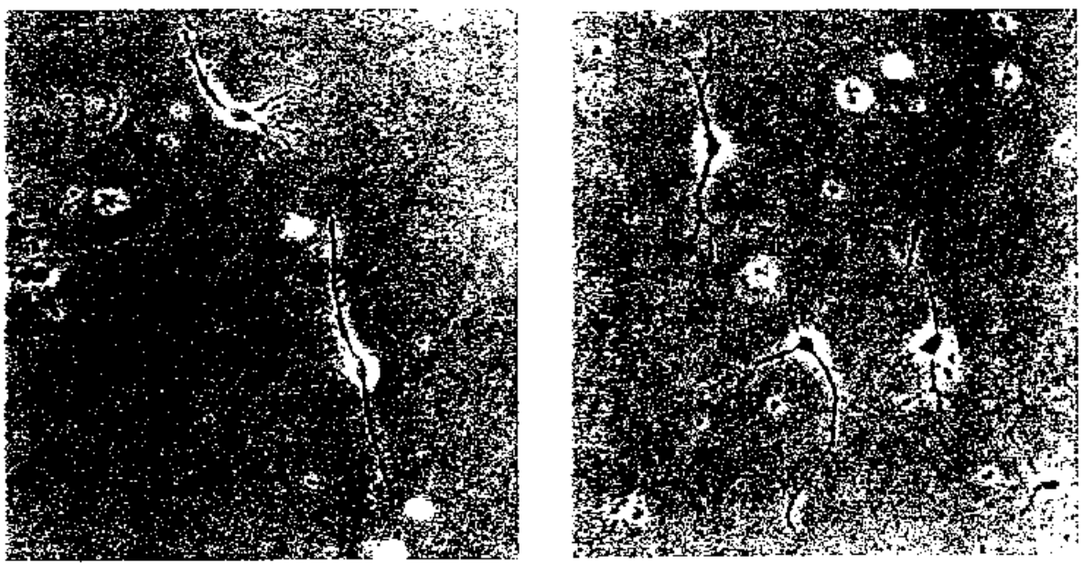
410
400
390
Binding Energy (eV)


C 1s
Normalized XPS Intensity
Calculated Film Thickness (angstroms)
Days
PDL w/cells
DETA w/cells
DETA no cells
0
2
4
6
8
10
12
0
31
42
50
56
60
64
67
69

ADULT NEURONS OF RAT CEREBRAL CORTEX CULTURED ON DETA IN SERUM-FREE MEDIUM

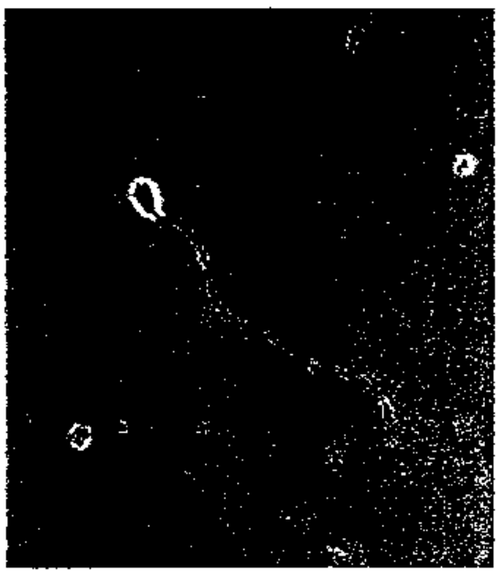


1-day-old culture



4-day-old cultures

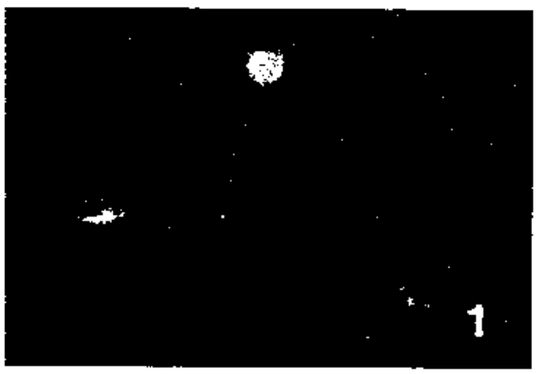
ADULT RAT CORTICAL NEURON CULTURED IN SERUM-FREE MEDIUM



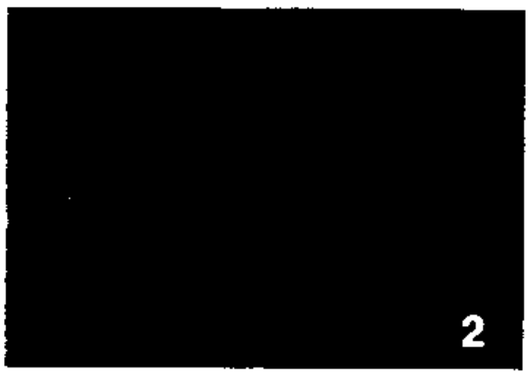
Phase-contrast

MAP2 immunostaining

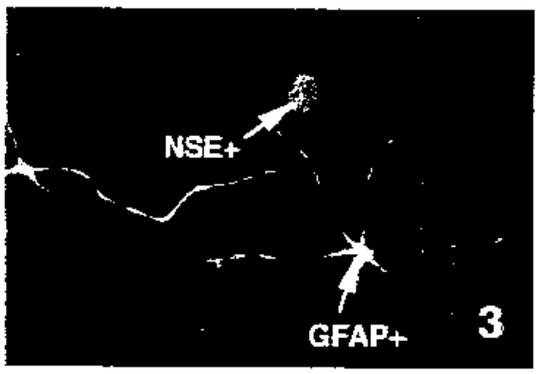
Survival of cerebral cortical cells in 21-day-old cultures



DETA



DETA



DETA + NT4

Poly-D-lysine + CNTF

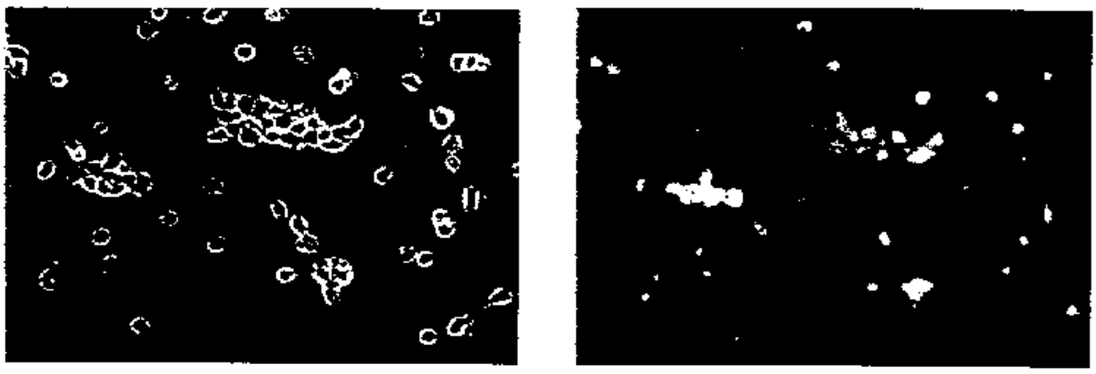
Poly-D-lysine

Poly-D-lysine + CNTF

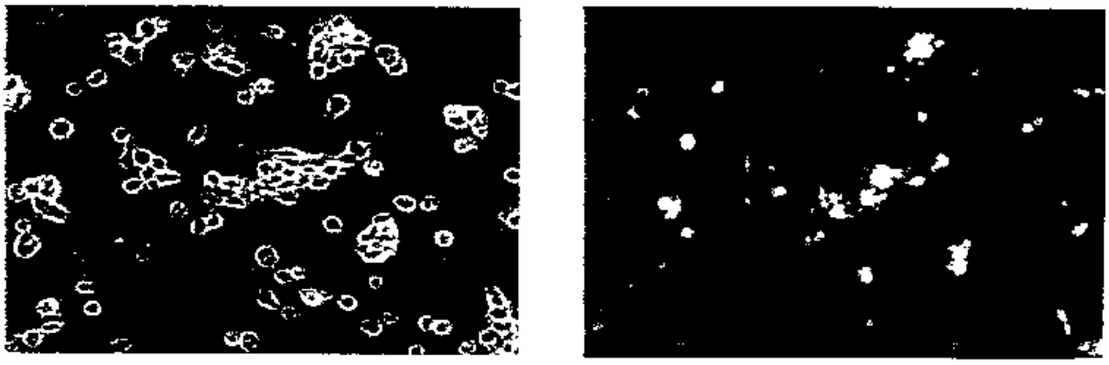
GABAergic/Glutamategic EXPRESSION IN CORTICAL CELLS CULTURED ON ARTIFICIAL SURFACES

Phase-contrast

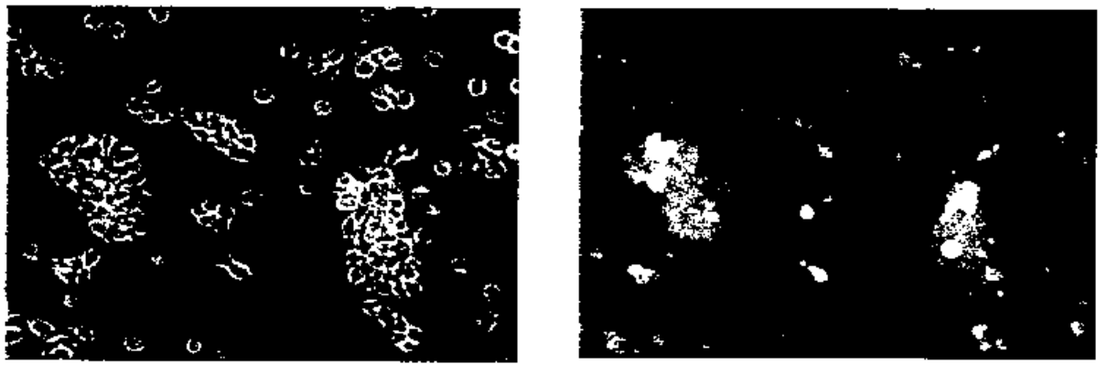
Double-fluorescence immunostaining



Poly-D-lysine

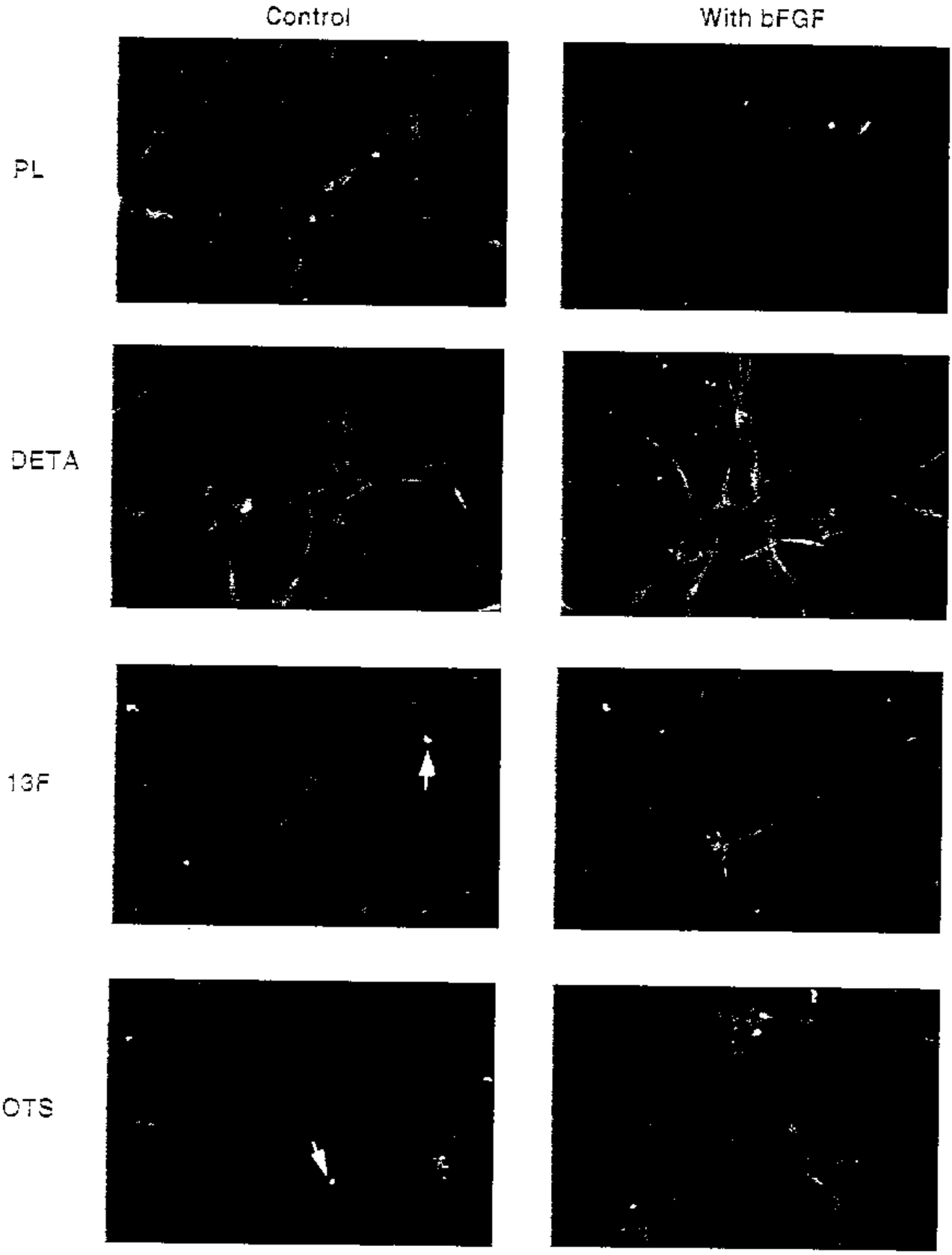


DETA

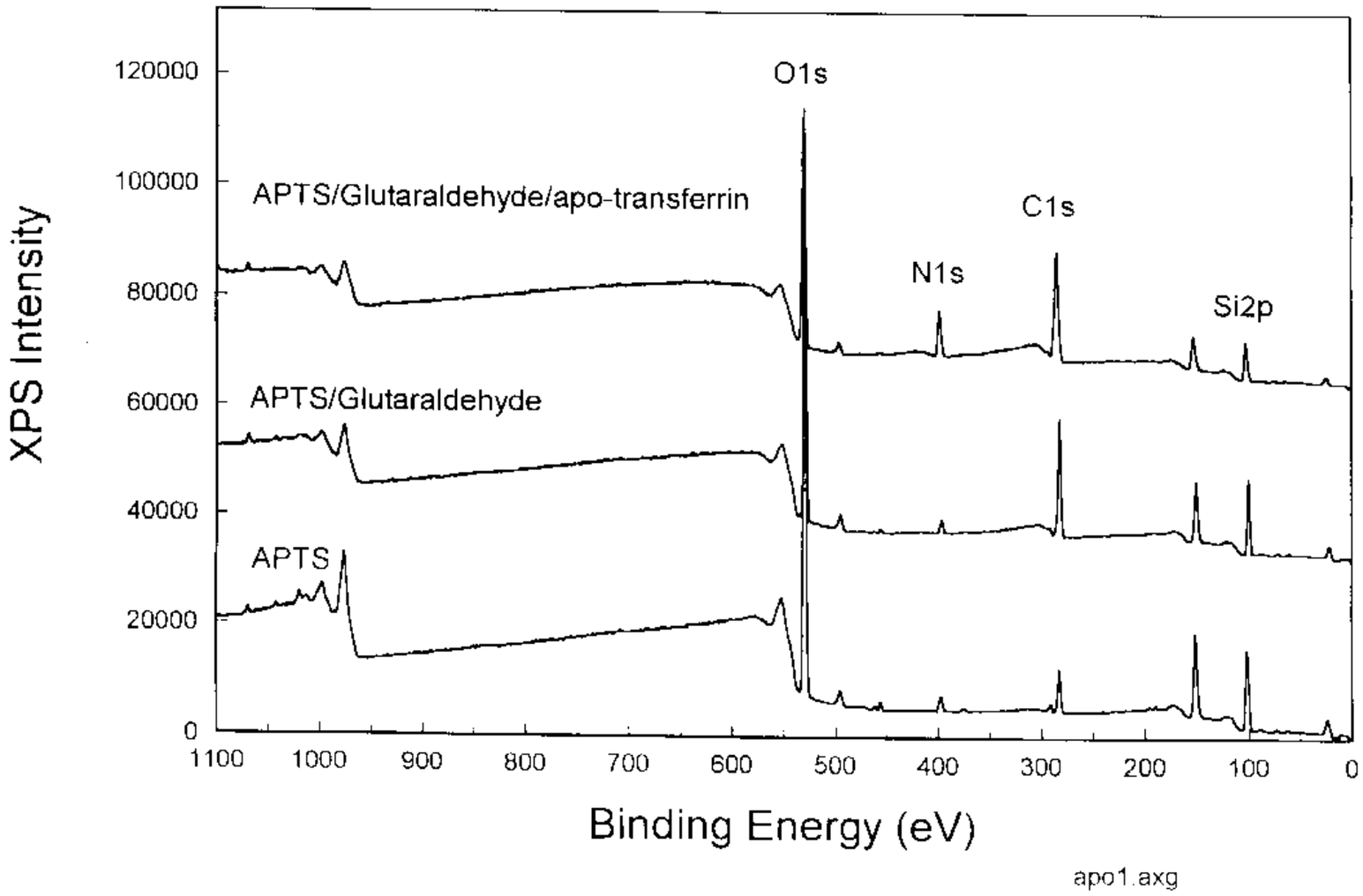


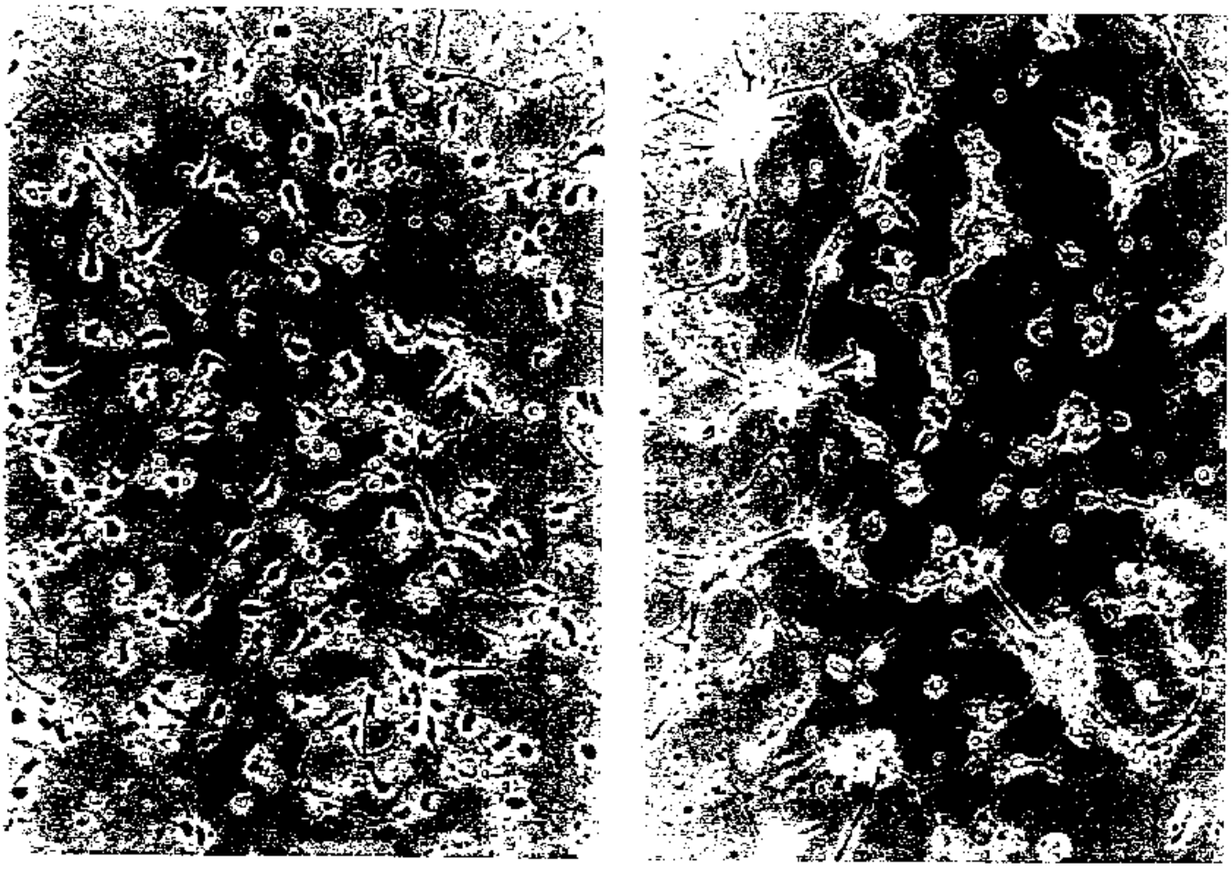
13F

4-DAY ASTROCYTIC CELL CULTURES IN SERUM-FREE MEDIUM



Attachment of Biomacromolecules to SAMs

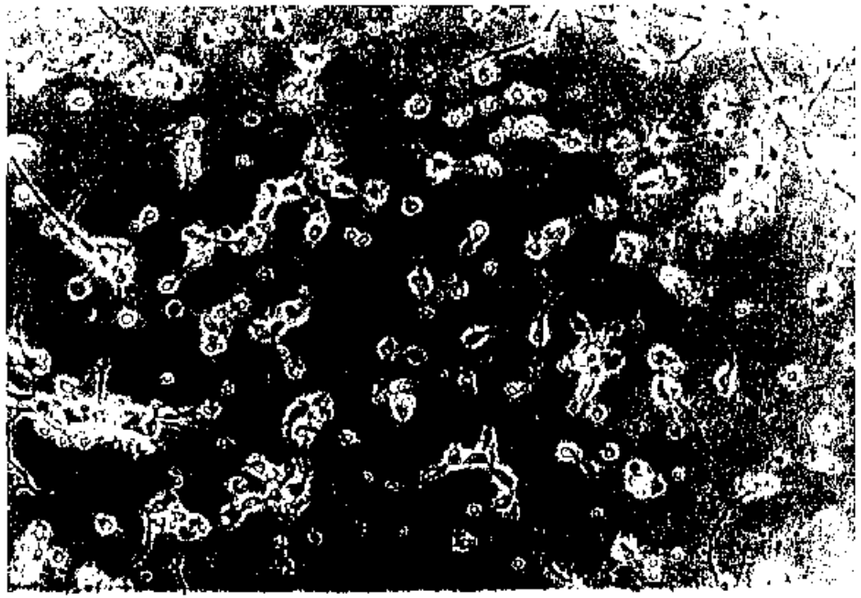




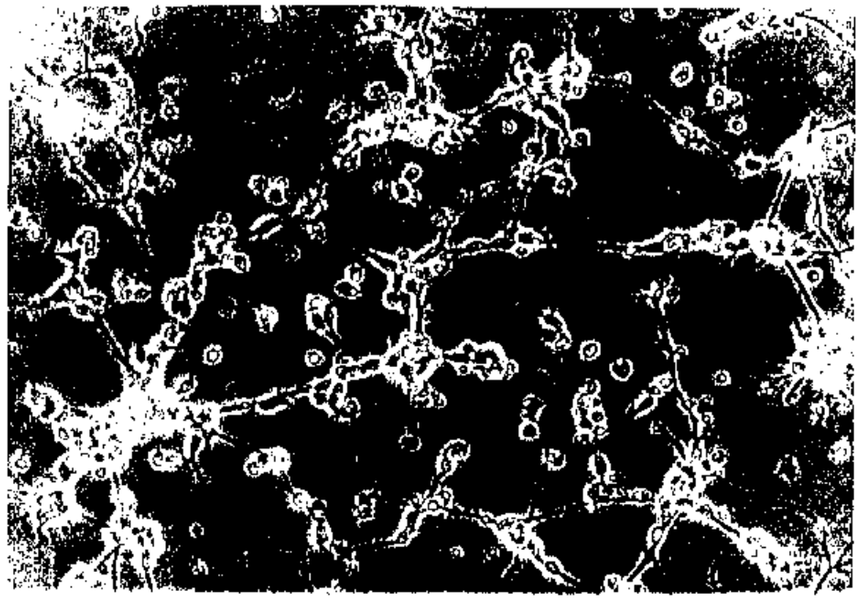
(a) PDL (b) APTS

(c) APTS + GLT

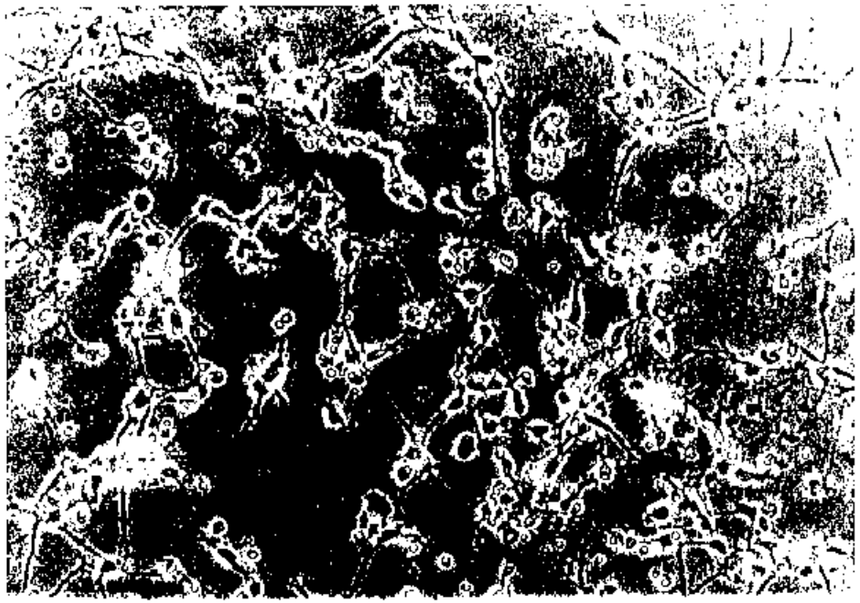
E18 RAT CORTICAL NEURONS ON BIOLOGICALLY MODIFIED SUBSTRATES



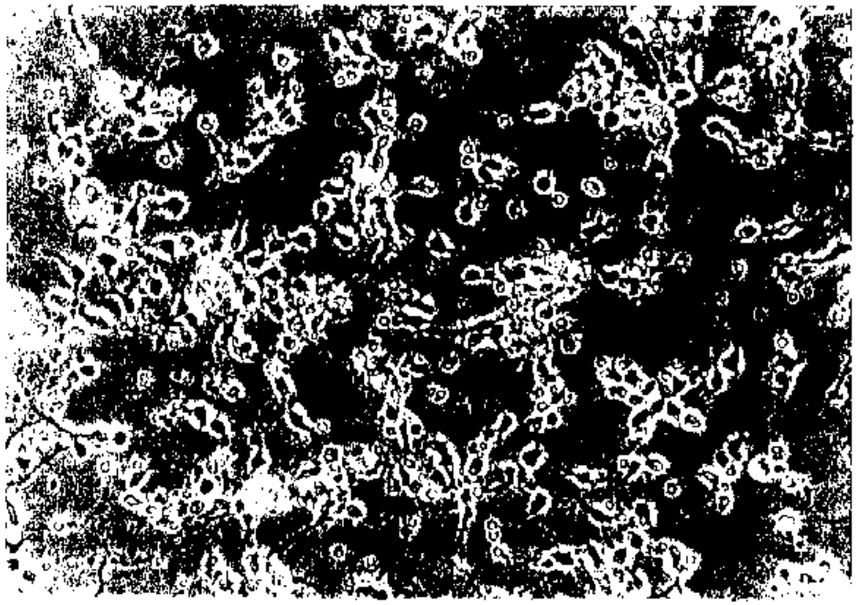
A — APTS + APO



B — APTS + GLUT + APO



C — APTS + BSA

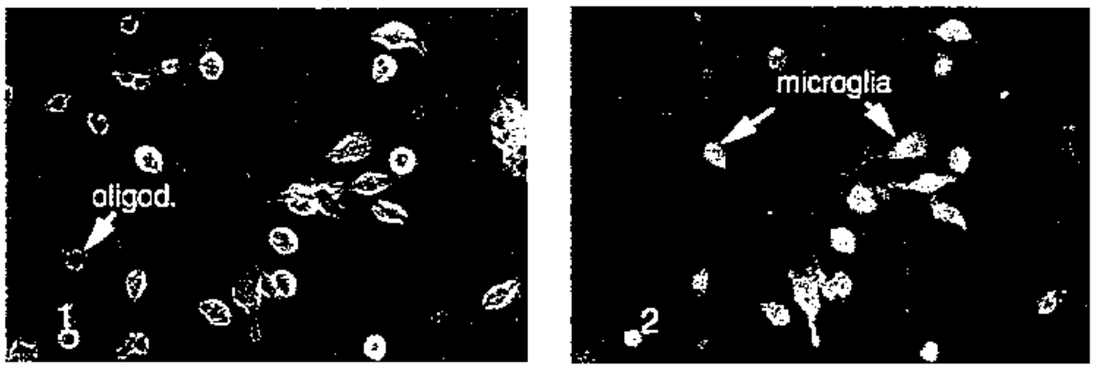


D — APTS + GLUT + BSA

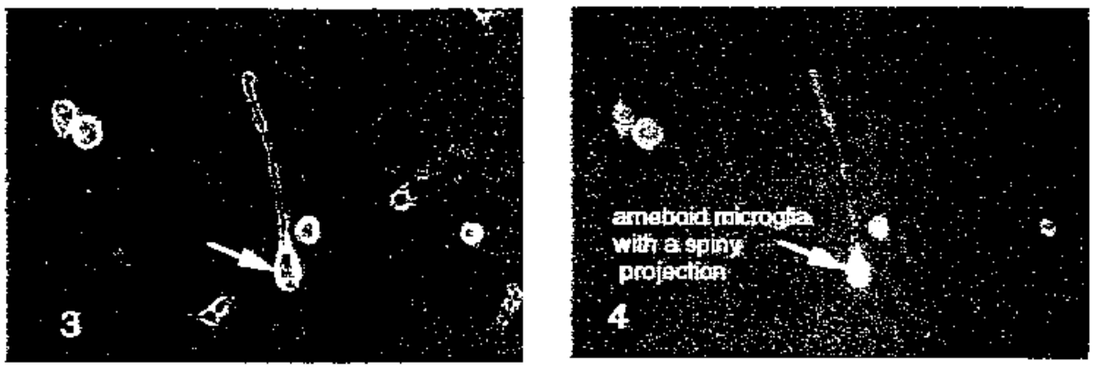
Enriched cultures of rat microglia derived from cerebral cortex

Phase-contrast

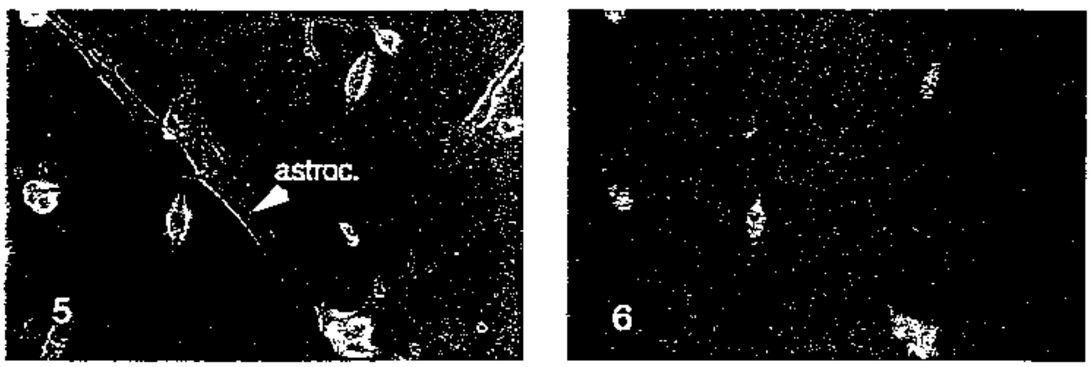
Immunostaining with Anti-OX42



Poly-D-Lysine



Poly-D-Lysine

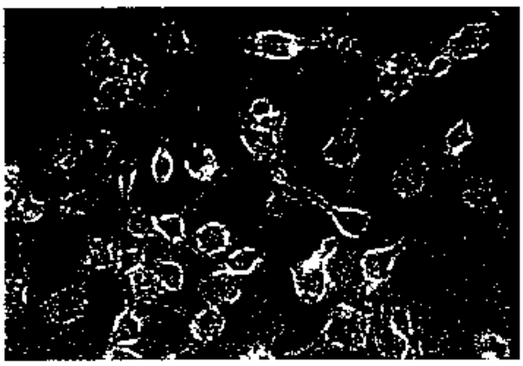


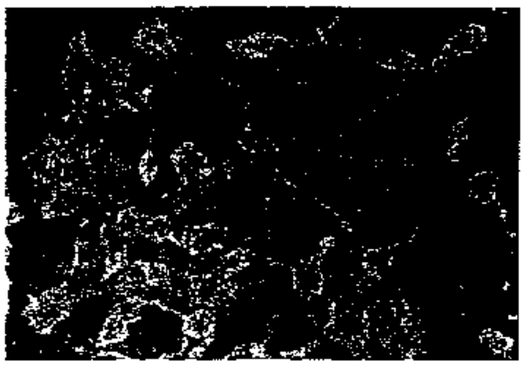
DETA

EFFECT OF SURFACES ON CORTICAL MICROGLIAL SURVIVAL AND GROWTH IN CULTURE

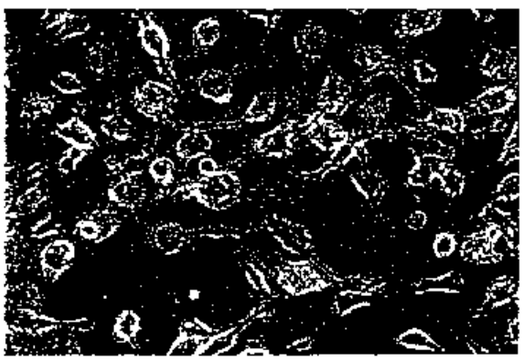
Phase-contrast

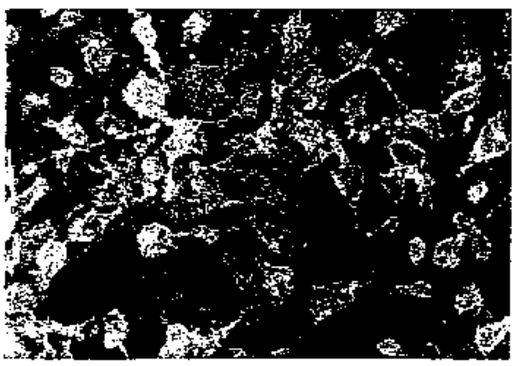
Anti-OX42



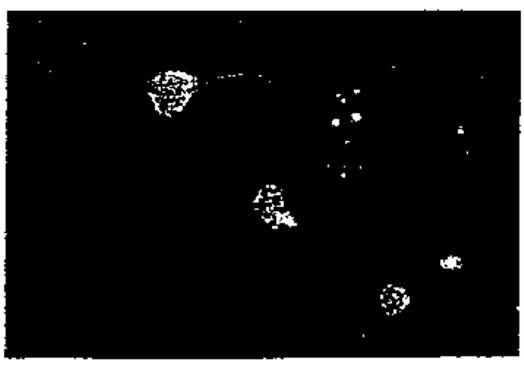


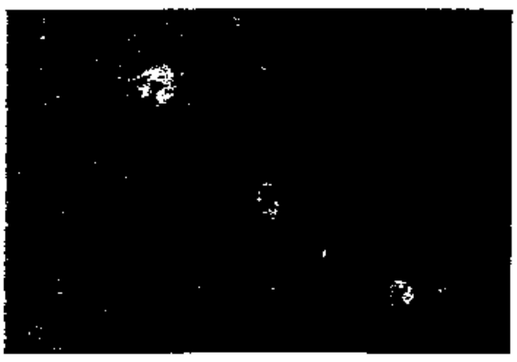
Poly-D-Lysine





DETA





13F